## **Comparison of the biological potential of luteolin before and after** enzymatic modification with *Thermomyces lanuginosus* lipase

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Introduction Luteolin is a common flavonoid that exists in many types of plants, such as fruits, vegetables and medicinal herbs. This compound has multiple biological effects such as anticancer, antioxidant and anti-inflammatory activity. As a flavonoid, luteolin presents several hydroxy- groups, which limits their applications in some fields, due to their low solubility in lipophilic systems. To avoid that, luteolin can be acylated by lipase, using vinyl ester as acyl-donor. The enzymatic method is more selective and occurs in mild reaction conditions, in comparison with classic chemical methods.

Aim The cytotoxicity evaluation of the non-modified luteolin and its comparison with the corresponding action of the reaction mixture after enzymatic modification with *Thermomyces lanuginosus* lipase (Luteolin-TLL) (3'-O-acetyl luteolin and 4'-O-acetyl luteolin), as well as the isolated fraction (3'-O-acetyl luteolin).

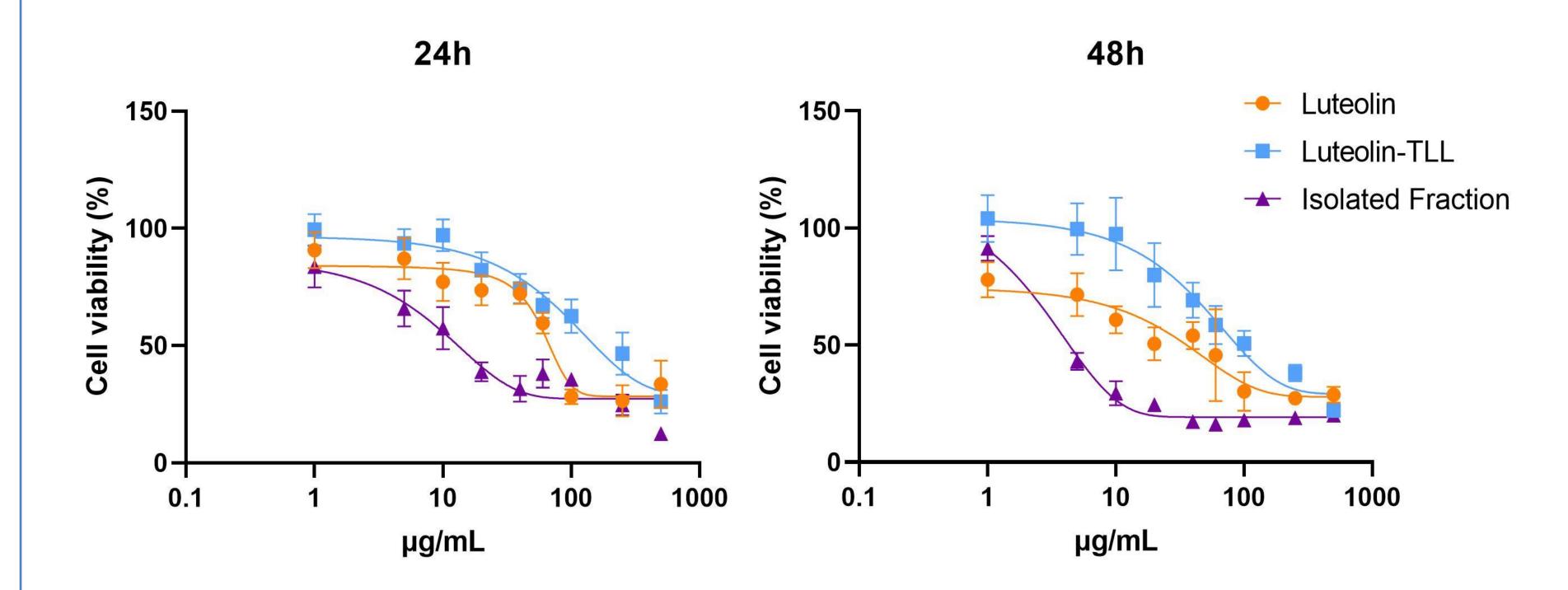
Methods The NIH/3T3 fibroblasts were used for the *in* vitro experiments. Cytotoxicity was estimated by means of the MTT and clonogenic assay and flow cytometry was applied for the detection of Reactive Oxygen Forms (ROS) and cell cycle analysis.



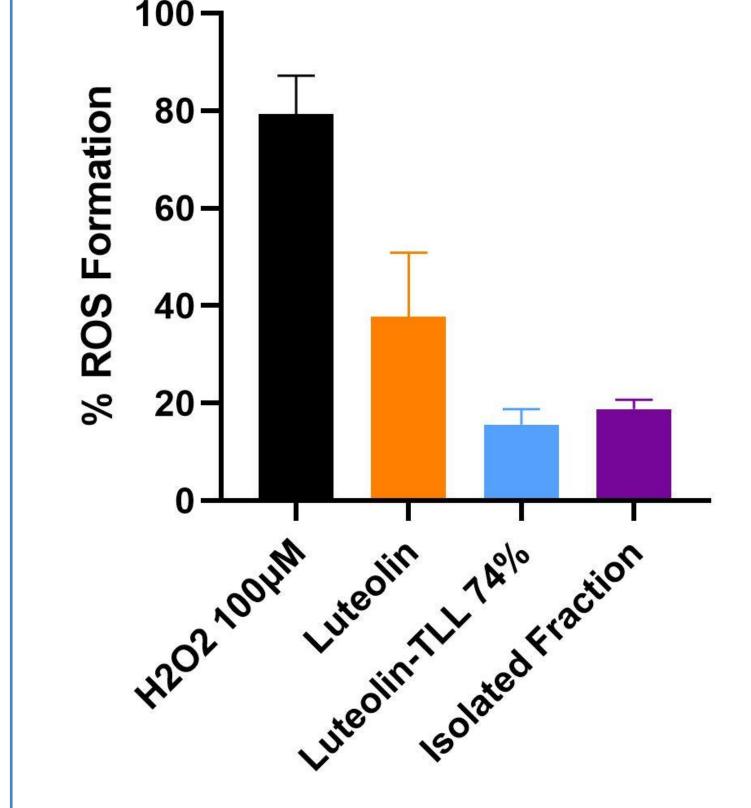


Πανεπιστήμιο

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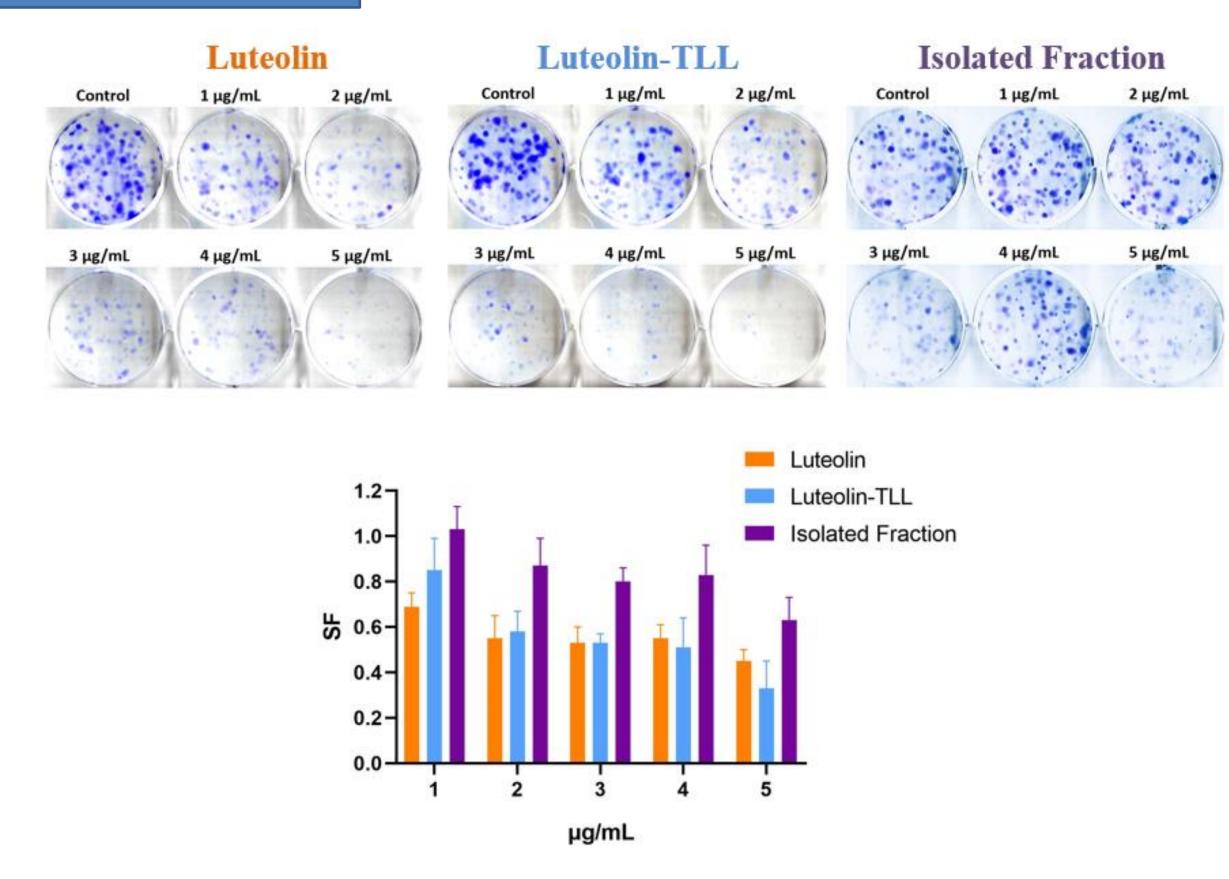
The compounds showed a dose- and time-dependent cytotoxic effect against NIH/3T3 cells. The mixture of modified luteolin (3'-O-acetyl luteolin and 4'-O-acetyl luteolin) showed significant lower cytotoxicity against NIH/3T3 cells, especially after 48h of treatment. On the contrary, the isolated fraction (3'-O-acetyl luteolin) appeared to be more toxic than both luteolin and the modified mixture.



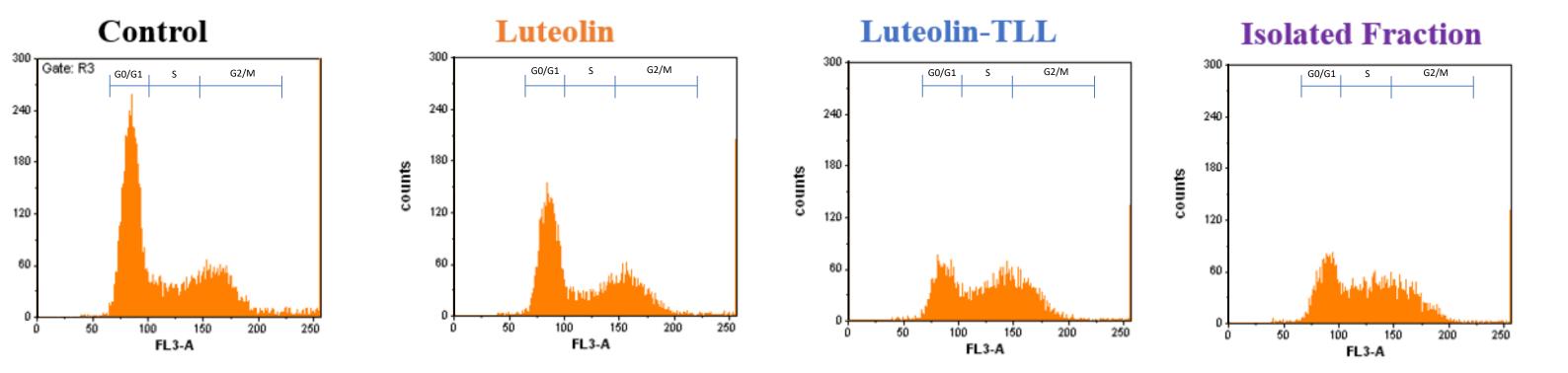
Treatment with 20  $\mu$ g/mL of the three compounds, that luteolin-TLL mixture and the isolated showed ability to scavenge presented a greater fraction intracellular ROS, reducing the formation of ROS by 63,7% and 60,5% respectively, in contrast to the isolated fraction.

## **Colonies Formation**

## **Cell Cycle Analysis**



Long-term toxicities of luteolin and luteolin-TLL mixture were higher than their short-term toxicities, while the isolated fraction with high short-term toxicity had lower long-term toxicity.



	G0/G1	S	Μ
Control	$\textbf{48,91} \pm \textbf{1,7}$	$\textbf{32,62} \pm \textbf{2,0}$	$\textbf{18,}\textbf{47} \pm \textbf{1,}\textbf{6}$
Luteolin	$\textbf{47,}\textbf{10} \pm \textbf{2,}\textbf{1}$	$\textbf{24,81} \pm \textbf{2,3}$	28,09 ±2,4
Luteolin-TLL	$\textbf{28,65} \pm \textbf{1,1}$	$43,55 \pm 2,0$	$\textbf{27,80} \pm \textbf{2,4}$
<b>Isolated Fraction</b>	$\textbf{28,21} \pm \textbf{1,9}$	$\textbf{47,}\textbf{17} \pm \textbf{1,}\textbf{5}$	$\textbf{24,62} \pm \textbf{2,1}$

Treatment with 10 µg/mL luteolin-TLL mixture or the isolated fraction resulted in a moderate increase in S-phase and a mild increase in M-phase. On the contrary, luteolin arrested proliferating NIH/3T3 cells at M-phase.

Conclusions Enzymatic modification with TLL differentiated luteolin's biological effects especially long-term cytotoxicity against the normal cells. Nonetheless, further molecular experiments will unfold more details about the compound's mechanism of action.

## **Bibliography**

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